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Chen-Yuan Dong, Christof Buehler, Peter T C So, and Enrico Gratton.

Two-photon time-resolved polarization imaging inside mouse fibroblast cells.

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Abstract

Two photon time-resolved microscopy has proven to be a powerful technique to image biological systems. The high incident photon flux from a high peak power light source, such as a mode-locked titanium sapphire laser, can be focused to a diffraction limited spot inducing effective two-photon excitation of the chromophores. The nonlinear nature of the two photon process results in a strong localization of the excitation volume, which minimizes photo-bleaching of the specimen. We combined the 3-D sectioning effect inherent in two photon microscopy with time-resolved methods to study rotational correlation times of fluorophores in living mouse fibroblasts. Images were acquired with a microscope in a point-by-point fashion by raster scanning a mode-locked titanium sapphire laser beam across the specimen. At each sampling point, time-resolved information is obtained by analyzing the sample frequency response to the laser harmonic content. The measurement of the decay of the emission anisotropy allowed us to probe the chromophore's micro-environment, revealing structural and dynamic properties inside living mouse fibroblast cells. Supported by the National Institutes of Health, RR03155.